Amendments to the Specification

Please replace the paragraph beginning at page 1, line 26 with the following amended paragraph:

On therapeutic use in the body, many proteins are very quickly removed from the bloodstream or degraded. Systemically administered proteins having a molecular weight of more than about 70 ~~kD~~ kDa may be removed from the circulation by the reticuloendothelial system or specific interactions with cellular receptors. Smaller proteins having a molecular weight of less than about 70 ~~kD~~ kDa may in addition be removed to a large extent by the glomerular filtration in the kidney (exclusion limit about 70 ~~kD~~ kDa).

Please replace the paragraph beginning at page 2, line 1 with the following amended paragraph:

An approach followed recently to eliminate the described problems consists in coupling such problematic proteins to biocompatible polymers with good solubility in water, such as, for example, polyethylene glycol and dextran. On the one hand, it is possible by the coupling to increase the molecular weight above the threshold of 70 ~~kD~~ kDa, so that the plasma residence time of smaller proteins can be drastically increased, and on the other hand the solubility in aqueous medium can be improved by the hydrophilic polymer portion.

Please replace the paragraph beginning at page 6, line 2 with the following amended paragraph:

The average molecular weight of the hydroxyalkylstarch can be in the range from about 3 ~~kD~~ kDa to several million daltons, preferably about 4 ~~kD~~ kDa to about 1000 ~~kD~~ kDa, more preferably in the range from about 4 ~~kD~~ kDa to about 50 ~~kD~~ kDa or in the range from about 70 ~~kD~~ kDa to about 1000 ~~kD~~ kDa, particularly preferably about 130 ~~kD~~ kDa. For coupling to small proteins, the average molecular weight of the hydroxyalkylstarch is preferably chosen so that the abovementioned threshold of 70 ~~kD~~ kDa is exceeded with the conjugates, whereas for coupling to large proteins the molecular weight of the hydroxyalkylstarch will preferably be in the lower region of said range. Since coupling is possible at a plurality of sites in a protein, it may also be

advantageous to couple a plurality of small polymer chains, instead of one of high molecular weight. The degree of substitution (ratio of the number of modified anhydroglucose units to the number of anhydroglucose units in total) may likewise vary and will frequently be in the range from about 0.2 to 0.8, preferably about 0.3 to 0.7, more preferably about 0.5. (Note: the numbers relate to the "degree of substitution", which is between 0 and 1). The ratio of $C_2$ to $C_6$ substitution is normally in the range from 4 to 16, preferably in the range from 8 to 12.

Please replace the paragraph beginning at page 7, line 4 with the following amended paragraph:

Hydroxyethylstarch products with an average molecular weight of 130 ~~kD~~ kDa and a degree of substitution of 0.5, and with an average molecular weight of 200 ~~kD~~ kDa and a degree of substitution of 0.25, have already been used clinically as blood substitutes and are also suitable for use in the present invention.

Please replace the paragraph beginning at page 20, line 15 with the following amended paragraph:

10 g of HES-130 ~~kD~~ kDa were dissolved in 12 ml of deionized water by heating in a round-bottomed flask. 2 ml of an 12 solution (0.1N) were added to this solution. A pipette with 2 ml of 1.0N NaOH was connected to the flask via a 2-way connector, and the NaOH solution was added dropwise at about 1 drop every 4 minutes. The solution was decolorized after addition of approximately 0.2 ml of the NaOH solution and, at this time, a second portion of 2 ml of 0.1N iodine solution was added. The reaction was complete after addition of a total of 14 ml of iodine solution and 2.8 ml of NaOH solution. The reaction mixture was then dialyzed against deionized water.

Please replace the paragraph beginning at page 21, line 17 with the following amended paragraph:

An approximately quantitative yield was achieved (>98%). It is possible by this procedure to oxidize hydroxyethylstarches with higher molecular weight (e.g. 130 ~~kD~~ kDa, 250

~~kD~~ kDa, 400 ~~kD~~ kDa) just like hydroxyethylstarches with lower molecular weight (e.g. 10 ~~kD~~ kDa, 25 ~~kD~~ kDa, 40 ~~kD~~ kDa), in similarly high yields.

Please replace the paragraph beginning at page 21, line 27 with the following amended paragraph:

A solution of 0.24 mmol of HES-130 ~~kD~~ kDa was prepared in 10 ml of deionized water with heating. This solution was heated in a 100 ml round-bottomed flask to a temperature of 70-80°C, and 1.17 mmol of stabilized $Cu^{2+}$ (e.g. Rochelle salt as stabilizer or other stabilizers) and dilute aqueous NaOH solution was added (final concentration 0.1N NaOH). The temperature was then raised to 100°C, and the reaction was allowed to proceed until a reddish color had appeared. The reaction was stopped and the reaction mixture was cooled to 4°C. The reddish precipitate was removed by filtration. The filtrate was dialyzed against deionized water and then converted into the lactone as in example 1 and lyophilized. The oxidation took place quantitatively (yield >99%). It was also possible by this method to oxidize low molecular weight HES (e.g. HES-10 ~~kD~~ kDa, HES-25 ~~kD~~ kDa, HES-40 ~~kD~~ kDa) and higher molecular weight HES species.

Please replace the paragraph beginning at page 22, line 9 with the following amended paragraph:

Coupling of Selectively Oxidized High Molecular Weight HES (ox-HES-130 ~~kD~~ kDa) to Human Serum Albumin (HSA)

Please replace the paragraph beginning at page 22, line 12 with the following amended paragraph:

4.3 g of ox-HES-130 ~~kD~~ kDa and 200 mg of HSA (Sigma, Taufkirchen) were completely dissolved in water by gentle heating in a round-bottom flask with magnetic stirrer. 30 mg of ethyldimethylaminopropylcarbodiimide (EDC), dissolved in water, were added to this solution. After stirring very moderately for 2 h, a second portion of 30 mg of EDC was added. After stirring very moderately for a further two hours, a third portion of 40 mg of the carbodiimide was added. The reaction mixture was left under these conditions overnight, dialyzed against distilled

water for 15 h and lyophilized. The success of the coupling was demonstrated by gel permeation chromatography, SDS-PAGE and carbohydrate-specific staining (Glyco-Dig kit from Roche-Boehringer, Basle) after blotting onto a PVDF membrane. The yield of coupling product was about 90%.

Please replace the paragraph beginning at page 22, line 29 with the following amended paragraph:

Coupling of Selectively Oxidized Low Molecular Weight HES (ox-HES-10 ~~kD~~ kDa) to Human Serum Albumin (HSA)

Please replace the paragraph beginning at page 22, line 32 with the following amended paragraph:

7.4 g of ox-HES-10 ~~kD~~ kDa and 50 mg of HSA were completely dissolved in water in a round-bottom flask with magnetic stirrer. The reaction was carried out by the method described above for high molecular weight HES, adding a total of 282 mg of EDC in three aliquots. The reaction mixture was likewise dialyzed and lyophilized as described above. Analysis (as above) showed the coupling product was obtained, but the yields were somewhat lower than in the coupling with high molecular weight ox-HES.

Please replace the paragraph beginning at page 23, line 7 with the following amended paragraph:

Coupling of ox-HES-130 ~~kD~~ kDa to Myoglobin (Mb)

Please replace the paragraph beginning at page 23, line 9 with the following amended paragraph:

4.3 g of ox-HES-130 ~~kD~~ kDa were completely dissolved in water (6-7 ml), and then 100 mg of Mb (Sigma, Taufkirchen), dissolved in 10 ml of 0.1 M phosphate buffer (pH 7.0), were added. The coupling reaction was started by adding 30 mg of EDC. Addition of EDC was repeated every 2 hours until a total of 90 mg of the carbodiimide had been consumed. The

reaction mixture was then dialyzed against 50 mM phosphate buffer, pH 7.0, and lyophilized. GPC showed a definite product peak, which was detected in the hold-up volume at 450 nm. It was possible to calculate a coupling yield of 88% from this. The oxygen-binding capacity of the hesylated myoglobin was about 76% of the binding capacity of unmodified Mb.

Please replace the paragraph beginning at page 23, line 24 with the following amended paragraph:

Coupling of ox-HES-10 ~~kD~~ kDa to Superoxide Dismutase (SOD)

Please replace the paragraph beginning at page 23, line 26 with the following amended paragraph:

One part by volume of an aqueous solution of ox-HES-10 ~~kD~~ kDa (1.05 g/ml) was incubated with one part by volume of a 7 mg/ml SOD solution (Sigma, Taufkirchen) in 50 mM phosphate buffer, pH 7.6, at room temperature. The coupling reaction was initiated by adding 280 mg of EDC in 5 portions over a period of 24 h. The progress of the reaction was followed by GPC analysis in phosphate buffer and detection at 280 nm. After 24 h, 81% of the protein were found in the higher molecular weight region of the separating column, and the reaction was stopped after this time. The reaction mixture was subjected to a diafiltration with a 30 ~~kD~~ kDa membrane and then lyophilized. Mass spectrometric analysis of the product showed an average molar ratio of HES to protein of about 3:1.

Please replace the paragraph beginning at page 24, line 6 with the following amended paragraph:

Coupling of ox-HES-130 ~~kD~~ kDa to Streptokinase (SK)

Please replace the paragraph beginning at page 24, line 8 with the following amended paragraph:

3.8 kg of ox-HES-130 ~~kD~~ kDa were dissolved together with 35 mg of streptokinase (Sigma, Taufkirchen) in the minimum amount of 50 mM phosphate buffer, pH 7.2. At room

temperature, 46.5 mg of EDC and 20 mg of 1-hydroxybenzotriazole hydrate (HOBt) were added, and reaction was maintained with gentle stirring for a total of 24 h. After dialysis and freeze drying, about 78% of the protein were found as HES conjugate by GPC analysis. In the SDS-PAGE with silver staining, a distinct increase in the molecular mass of the streptokinase was observable. In parallel with this, carbohydrate structures were unambiguously detectable in the high molecular waveband with the digoxigenin method.

Please replace the paragraph beginning at page 24, line 22 with the following amended paragraph:

Coupling of ox-HES-130 ~~kD~~ kDa to Human Interleukin-1 (IL-2)

Please replace the paragraph beginning at page 24, line 24 with the following amended paragraph:

45 mg of ox-HES-130 ~~kD~~ kDa were completely dissolved in 0.5 ml of 50 mM Na phosphate buffer, pH 6.5, with gentle heating. After addition of 0.25 mg of human IL-2 (Sigma, Taufkirchen), which made the solution opaque, the mixture was stirred at room temperature for 4-6 h. Then 5 mg of EDC were added in 4 portions with a time difference of 2 h for each, and stirring was continued overnight, resulting in a clear solution. GPC analysis revealed a coupling yield of about 65%.

Please replace the paragraph beginning at page 25, line 2 with the following amended paragraph:

Coupling of ox-HES-25 ~~kD~~ kDa to Human Tumor Necrosis Factor α (TNFα)

Please replace the paragraph beginning at page 25, line 5 with the following amended paragraph:

0.3 mg of hTNFα (Sigma, Taufkirchen) were added to 86 mg of ox-HES-25 ~~kD~~ kDa in about 0.4 ml of 0.1 M phosphate buffer (pH 7.0). The cloudy solution was stirred for about 2 h before 1 mg of EDC and 0.5 mg of HOBt were added. Stirring was continued for about 6 h, with

the solution becoming clear during the reaction time. The coupling product was isolated by ultrafiltration and freeze drying and analyzed by GPC and detection at 280 nm. A coupling yield of approximately 74% was found in this case.

Please replace the paragraph beginning at page 25, line 16 with the following amended paragraph:

Coupling of ox-HES-130 ~~kD~~ kDa to Glucagon-Like Peptide (GLP-1)

Please replace the paragraph beginning at page 25, line 18 with the following amended paragraph:

7.4 g of ox-HES-130 ~~kD~~ kDa were dissolved in a minimum volume of water by heating and gentle stirring. A solution of 10 mg of GLP-1 in the amide form (Bachem, Switzerland) in 50 mM phosphate buffer, pH 7.4, was added by pipette. The reaction was started by adding 35 mg of EDC and was cautiously stirred for 2 h. This was repeated 2x more because, after this time, a peptide peak was no longer evident in the GPC analysis at 280 nm, i.e. approximately complete conversion to the coupling product had taken place. This coupling product was diafiltered using a 30 ~~kD~~ kDa membrane and lyophilized from phosphate buffer solution. It was possible to conclude from the results of a MALDI mass spectroscopy that the stoichiometry between peptide and HES was 1:1.

Please replace the paragraph beginning at page 26, line 2 with the following amended paragraph:

Coupling of High Molecular Weight HES (HES-130 ~~kD~~ kDa) to Human Serum Albumin (HSA)

Please replace the paragraph beginning at page 26, line 5 with the following amended paragraph:

9.75 g of HES-130 ~~KD~~ kDa were completely dissolved in water (6-7 ml), and then 50 mg of HSA, dissolved in 1 ml of 0.1 M phosphate buffer (pH 7.4) were added. The reaction mixture

was stirred with a magnetic stirrer. The solution was then mixed with $NaBH_3CN$ (50-70 mg) and stirred gently for a few minutes. The solution was further stirred for 15 minutes every two hours. Then a further aliquot of $NaBH_3CN$ (about 50 mg) was added. At the end (after a reaction time of almost 36 h), a total amount of 285 mg of $NaBH_3CN$ had been employed. The solution was then dialyzed and lyophilized. Analysis took place as described in example 4. The coupling efficiency was about 65%.

Please replace the paragraph beginning at page 26, line 19 with the following amended paragraph:

Coupling of Low Molecular Weight HES (HES-~~130 kD~~ -10 kDa) to Human Serum Albumin (HSA)

Please replace the paragraph beginning at page 27, line 2 with the following amended paragraph:

Coupling of HES-40 ~~kD~~ kDa to Asparaginase

Please replace the paragraph beginning at page 27, line 4 with the following amended paragraph:

3.0 g of HES-40 ~~kD~~ kDa were completely dissolved in water (about 4 ml). A solution of 80 mg of asparaginase (Sigma, Taufkirchen) in 6 ml of 0.1 M borate buffer, pH 9.0, were added thereto and stirred until the reaction mixture was clear. The temperature was then raised to 37°C. and, after 2 h, about 50 mg of $NaBH_3CN$ were added. This reaction cycle was repeated 3x. more. The product was worked up by dialyzing the reaction mixture against 0.1 M phosphate buffer, pH 7.4. The yield of coupling product was about 61%, and about 73% of the asparaginase activity was recoverable.

Please replace the paragraph beginning at page 27, line 17 with the following amended paragraph:

Coupling of HES-130 ~~kD~~ kDa to Human Interleukin-2 (IL-2)

Please replace the paragraph beginning at page 27, line 19 with the following amended paragraph:

50 mg of HES-130 ~~kD~~ kDa were completely dissolved in water (about 0.2 ml). A suspension of 0.25 mg of human IL-2 (Sigma, Taufkirchen) in 0.2 ml of 0.1 M borate buffer, pH 9.0, was added thereto and stirred until the reaction mixture was clear (4 h). 1 mg portions of $NaBH_3CN$ were added at intervals each of 4 h, and stirring was continued. After a further reaction time of 24 h, the mixture was dialyzed against 0.1 M phosphate buffer, pH 7.4 and lyophilized. The yield of coupling product was about 42% according to GPC analysis.

Please replace the paragraph beginning at page 27, line 31 with the following amended paragraph:

Coupling of HES-130 ~~kD~~ kDa to Insulin

Please replace the paragraph beginning at page 27, line 33 with the following amended paragraph:

4.0 g of HES-130 ~~kD~~ kDa were completely dissolved in water (about 6 ml). 55 mg of insulin from bovine pancreas (Sigma, Taufkirchen) in 7.5 ml of 0.1 M borate buffer (pH 9.0), were added thereto and stirred at 37°C. for about 24 h. The reducing agent $NaBH_3CN$ (60 mg in 30 ml) was slowly added dropwise over a period of 8 h. The reaction mixture was then stirred for a further 24 h and freed of faults and unreacted reagents by ultrafiltration (30 ~~kD~~). Lyophilization resulted in a stable coupling product. About 55% of the insulin employed was recovered as HES conjugate.

Please replace the paragraph beginning at page 28, line 9 with the following amended paragraph:

Coupling of ox-HES-130 ~~kD~~ kDa to Superoxide Dismutase (SOD)

Please replace the paragraph beginning at page 28, line 11 with the following amended paragraph:

130 mg of ox-HES-130 ~~kD~~ were completely dissolved in 6 ml of PBS pH 6, and then 10 mg of SOD (Roche, Mannheim) dissolved in 1 ml of PBS pH 6 were added. The coupling reaction was started by adding 10 mg of EDC. Addition of EDC was repeated every 3 h until 39 mg of the carbodiimide had been consumed. The reaction was monitored by GPC at 258 nm. After 24 h, 50% of the protein were found in the high molecular weight region of the separating column, and the reaction was stopped. The reaction mixture was dialyzed against 25 mM phosphate buffer pH 7.2 and lyophilized. The SOD activity was 95% of the initial activity. Determination of the mass distribution of HES protein samples by coupled GPC-light scattering revealed a molar ratio of HES to protein of 1:1.

Please replace the paragraph beginning at page 28, line 27 with the following amended paragraph:

Coupling of ox-HES 70 ~~kD~~ kDa to Glucagon

Please replace the paragraph beginning at page 28, line 29 with the following amended paragraph:

Glucagon ($66 \times 10^{-9}$ mol, 0.23 mg), oxHES 70 ~~kD~~ kDa ($6.6 \times 10^{-6}$ mol, 123 mg) were dissolved in phosphate buffer (1 ml, pH 5) in a round-bottom flask. 26 mg of EDC were added in 10 portions at intervals of 1 h. After a reaction time of 24 h, the reaction was stopped by adding 10 ml of water. The coupling product was purified by after dialysis against water by GPC and ion exchange chromatography. Freeze drying resulted in 88 mg of white coupling product (73%).

Please replace the Abstract with the following amended Abstract:

The invention relates to a method for coupling proteins to a starch-derived modified polysaccharide. The binding interaction between the modified polysaccharide and the protein is based on a covalent bond which is the result of a coupling reaction between the terminal aldehyde group or a functional group of the modified polysaccharide molecule resulting from the

chemical reaction of this aldehyde group and a functional group of the protein which reacts with the aldehyde group or with the resulting functional group of the polysaccharide molecule. The bond directly resulting from the coupling reaction can be optionally modified by a further reaction to the aforementioned covalent bond. The invention further relates to pharmaceutical compositions that comprise conjugates formed in this coupling process and to the use of ~~said~~ conjugates and compositions for the prophylaxis or therapy of the human or animal body.